RESEARCHREPORT

SWINE HEALTH

Title: Studies of the Viral Etiology of Porcine Postweaning Multisystemic Wasting Syndrome (PWMS).- **NPB #98-201**

Author: Steven Krakowka, DVM, PhD, Professor

Institution: The Department of Veterinary Biosciences, College of Veterinary Medicine, The Ohio State University, 1925 Coffey Road, Columbus, OH, 43210. Tel: 6140292-0231/ FAX: 614-292-6473, Email: krakowka.1@osu.edu

Date Received: 5/12/1999

Objective: The objectives of these studies was to determine the virulence of plaque-purified high titered Porcine circovirus (PCV)-1, PCV-2 and porcine parvovirus (PPV), alone or in combination in neonatal gnotobiotic swine and to confirm the hypothesis that overwhelming PCV-2 infection is the etiologic agent of porcine postweaning multisystemic wasting syndrome (PMWS).

Summary (from abstract of: Krakowka, S., Ellis, J. A., Meehan, B., Kennedy, S., McNeilly, R., and Allan, G. A.: Viral Wasting Syndrome of Swine: Experimental Reproduction of the Disease by Co-infection with Porcine Circovirus-2 (PCV-2) and Porcine Parvovirus (PPV), Vet Pathol., submitted for publication, 1999).

Groups (n=3-4) of one-day old gnotobiotic piglets (Table 1) from a total of 3 gnotobiotic litters of mixed breed piglets, housed in separate isolation units by infection group, were inoculated intranasally with plaque-purified viruses porcine circovirus (PCV)-1, PCV-2 and porcine parvovirus (PPV) alone or in combination (PCV-1/PCV-2, PCV-1/PPV and PCV-2/PPV) and then evaluated for the development of porcine postweaning multisystemic wasting syndrome (PMWS).

Single agent-infected piglets were clinically asymptomatic, transiently viremic and seroconverted to homologous virus. Microscopic lesions consisted of lymphoid hyperplasia and germinal center formation in lymphoid tissues and multifocal lymphocytic and plasmacytic inflammatory cell infiltrates in liver and myocardia.

Disease production in dually infected piglets was dependent upon the inocula used. Piglets inoculated with PCV-1/PCV-2 responded to infection by developing a pattern of disease indistinguishable from single agent (PCV-1 or PCV-2 alone) infection groups. Three of 4 seroconverted to PCV-1 and 4 of 4 were seropositive to PCV-2). Three of 4 PCV-1/PPV-inoculated piglets were clinically asymptomatic following infection which

was confirmed by serology: 4 of 4 seropositive to PCV-1 and 4 of 4 seropositive to PPV. In the remaining piglet of this dual infection group, subserosal and lamina proprial lymphangiectasia, most pronounced in the spiral and descending colon with associated granulomatous inflammation, was present. Unlike the other members of this infection group, PPV viremia was prolonged from 7 to at least 14 days after infection and very high titers to PCV-1 were detected in convalescent serum.

All 4 PCV-2/PPV inoculated piglets developed wasting disease characterized by icterus, submucosal edema, elevated serum liver enzymes and high (3 of 4) mortality rate. Lymph nodes in these piglets were reduced in size and the livers were mottled and discolored. Disseminated angiocentric granulomatous inflammation was present in all tissues examined except the brain. In lymph nodes and occasionally other tissues, multiple lightly basophilic intracytoplasmic inclusion bodies were identified in infiltrating macrophages and histiocytes. In the liver, variable to widespread, diffuse, moderate to severe hepatocellular necrosis and associated bile retention, closely associated with PCV-2-antigen-positive infiltrating macrophages was present. In these animals, liver failure was identified as the proximate cause of death. By immunocytochemistry, PCV-2 antigen was widely distributed in virtually every tissue and largely restricted to macrophages and histiocytes. Terminal sera from these piglets contained antibodies to PPV (4 of 4) and PCV-2 (3 of 4); the seronegative piglet had the most severe lesions and viral antigen was readily detected in peripheral blood mononuclear cells (PBMC).

The polymerase chain reaction (PCR) was used to track developing viremia (Table 2) and virus in secretions and excretions (Table 3). Piglets inoculated with PCV-1 alone or when combined with PPV were transiently viremic on postinfection day (PID) 7 only; piglets given PCV-1/PCV-2 remained negative for PCV-1. Parvovirus-infected piglets were viremic on PID 7 only except for the piglet with lamina proprial granulomatous inflammation which was viremic on PID 7 and 14. In contrast, one or all of the PCV-2-infected piglets, in each infection group remained viremic throughout the observation period. Cytospin preparations of isolated PBMC collected at termination from 2 of 4 piglets inoculated with PCV-2/PPV stained positively for PCV-2. Both PPV and PCV DNAs was identified in ocular, fecal and nasal secretions. These experiments confirm the hypothesis that disseminated PCV-2 infection is the cause of porcine PMWS. The mechanism(s) whereby other viral infectious co-factors (PPV) potentiate PCV-2 infection remains to be determined.

Table 1. A Summary of Results in Gnotobiotic Piglets Inoculated with Virus(es) Recovered from
Postweaning Multisystemic Wasting Syndrome (PMWS).

Piglet Group	Viral Inoculum	Morbidity and Infection	Gross Lesions	Microscopic Inflammatory
Control Group				
(n=3)	PK-15 cells alone	0/3[a]	none	none
Single Agent Infection Groups				
(n=3)	PCV-1	0/3	0/3	0/3
(n=3)	PCV-2	0/3	0/3	3/3
(n=3)	PPV	0/3	0/3	2/3[b]
Dual Agent Infection Groups				
(n=4)	PCV-1/PPV	0/4	1/4[c]	4/4
(n=4)	PCV-2/PPV	3/4	4/4	4/4
(n=4)	PCV-1/PCV-2	0/4	0/4	4/4
(n=2)	PCV-1/PCV-2 (contact)	0/2	0/2	0/2

[a] Number positive for (numerator)/ number evaluated

[b] Microscopic lesions consisted of focal lymphocytic cellular infiltrates in the liver and myocardium

[c] One of 4 PCV-1/PPV-infected piglets developed granulomatous inflammation of the intestinal lamina propria and associated lymphatics, especially of the spiral colon.

Table 2. A Summary of PCR for Viral DNAs in Porcine Peripheral Blood Mononuclear Cells and/or Whole Blood from Gnotobiotic Piglets Infected with Various Combinations of PCV-1, PCV-2 and PPV.

Piglet Infection Group	Post-infection Days (PID): 0	7	14	21	27/29	terminal or PID 35
Uninfected	-[a]	-	-	-	-	-
PCV-1	-	2/3[b]	-	-	-	-
PCV-2	-	-	2/3	3/3	3/3	2/3
PPV	-	2/3	-	-	-	-
PCV-1/PPV	-	3/4, 2/4[c]	1/4, 1/4[d]	-	-	-
PCV-2/PPV	-	- , 1/4	4/4, -	4/4, -	3/3, -	4/4, -[e]
PCV-1/PCV-2	-	-	- , 2/4	-, 3/4	-, 2/4	- , 1/4

[a] (-) the pooled (PID 0) sample tested negative for either viral DNA. Individual samples (PID 7 - 35) from uninfected controls all tested negative for viral DNAs; all samples tested at individual PID intervals tested negative for viral DNAs.

[b] Number positive for viral DNA (numerator)/ number tested (denominator)

[c] Number positive for PCV DNA (first fraction), number positive for PPV DNA (second fraction).

[d] The piglet which developed intestinal lymphangiectasia was PPV viremic on PID 7 and 14.

[e] In this piglet infection group (PCV-2/PPV), one piglet died on PID 27 and the remaining 3 piglets were terminated on PID 30.

Table 3. A Summary of PCR results for the Presence of Viral DNAs in Various Excretions and Secretions Collected at Termination from Gnotobiotic Piglets Infected with Various Combinations of PCV-1, PCV-2 and PPV.

Piglet Infection Group	Source of Materials: Fecal	Ocular	Nasal
Uninfected	-[a]	-	-
PCV-1	3/3[b]	3/3	2/3
PCV-2	2/3	3/3	1/3
PPV	-[a]	1/3	1/3
PCV-1/PPV	4/4, -[c]	4/4, 4/4	4/4, 2/4
PCV-2/PPV	3/3, -	3/3, -	3/3, 2/3[d]
PCV-1/PCV-2	-, 2/4	- , 3/4	- , -

[a] (-) the pooled sample from the uninfected controls tested negative for either viral DNA.

[b] Number positive for viral DNA (numerator)/ number tested (denominator)

[c] Number positive for PCV DNA (first fraction); number positive for PPV DNA (second fraction).

[d] In this piglet infection group (PCV-2/PPV), one piglet died on PID 27 and the remaining 3 piglets were terminated on PID 30.

Publications supported exclusively or in part by the NPPC, 1999

Papers supported directly by NPPC

1. Krakowka, S., Ellis, J. A., Rings, M., Allan, G., McNeilly, F. and Meehan, B.: Porcine Circovirus Infection: Reproduction of Postweaning Multisystemic Wasting Syndrome (PMWS) in Gnotobiotic Swine. Proc, Am Assn Swine Pract, 30th mtg, St Louis, MO, 30:417-422,1999.

2. Krakowka S, Ellis JA, McNeilly F, Meehan B, Rings M, Allan G: Porcine circovirus and the etiology of postweaning multisystemic wasting syndrome (PWMS). LCI Annual Mtg, Nashville, TN, 1999.

3. Ellis, J. A., Krakowka, S., Allan, G., Clark, E. and Kennedy, S.: The Clinical Scope of PRRSV Infection has Expanded Since 1987: An Alternative Perspective (letter to the editor), Vet Pathol, in press, 1999.

4. Krakowka, S., Ellis, J. A., Meehan, B., Kennedy, S., McNeilly, R., and Allan, G. A.: Viral Wasting Syndrome of Swine: Experimental Reproduction of the Disease by Co-infection with Porcine Circovirus-2 (PCV-2) and Porcine Parvovirus (PPV), Vet Pathol., submitted for publication, 1999.

Papers which were indirectly supported in part by the NPPC

1. Allan, G. M., Kennedy, S., McNeilly, F. Foster, J. C., Ellis, J., Krakowka, S., Meehan, B. and Adair, B.: Experimental Reproduction of Wasting Disease and Death by Co-infection of Piglets with Porcine Circovirus and Porcine Parvovirus. J. Comp. Pathol, in press, 1999.

2. West KH, Bystrom J, Wojanarowicz C, Allan G, Meehan B, Haines DM, Krakowka S, Clark EG, Kennedy S, Konoby C, Martin K and Ellis JA: Myocarditis and abortion associated with intrauterine infection of sows with porcine circovirus-2. J Vet Diagn Invest, submitted, 1999.

3. Ellis JA, Krakowka S, Allan G, Clark EG, Haines DM, West KH and Martin K: The Phenotype of Polykaryons in Lymph Nodes from Pigs with Naturally Aquired Porcine Circovirus-2 Infections. In preparation, 1999.

4. Krakowka, S., Ellis, J. A., Allan, G. A., West, K., McNeilly, F., Meehan, B. and Perkins,
W. E.: Evidence for Transplacental Transmission of Porcine Circovirus-2 Infection in Swine. In preparation, 1999.